Using Bioassays to Evaluate the Performance of Risk Management Techniques

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Common Risk Management Assumptions

- Risk - characterized by contaminants - Ignores
  - Incomplete removal or side products
  - Co-Contaminants
  - Matrix Effects
- Treatment reduces toxicity - Ignores
  - Process Amendments
  - Other reactions
  - Matrix Changes

Case Study 1

- Remediation of PCB Contaminated Soil by Solvent Extraction
- Principals: Mark Meckes, John Meier, and Lina Chang
- More information - Meier, et al. 1997. Environmental Toxicology and Chemistry, p. 928 - 938.

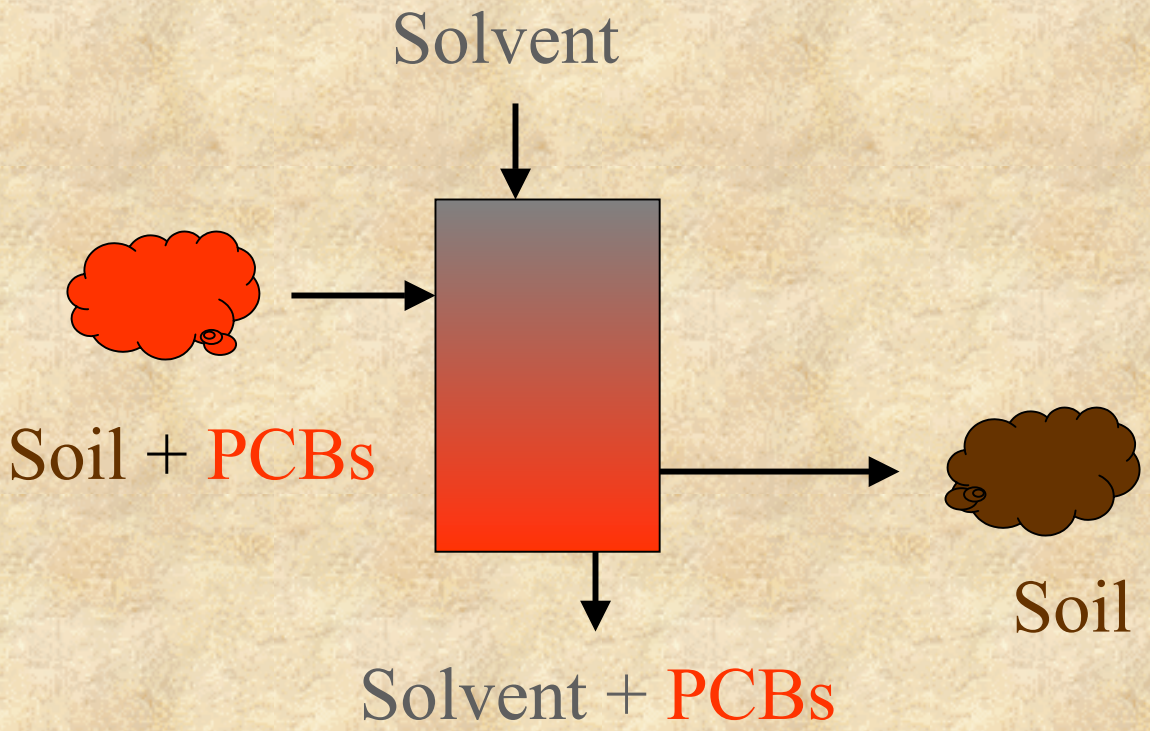
Case Study 1 - Chemical Analysis

- PCBs
- VOCs
- SVOCs
- Metals by TCLP

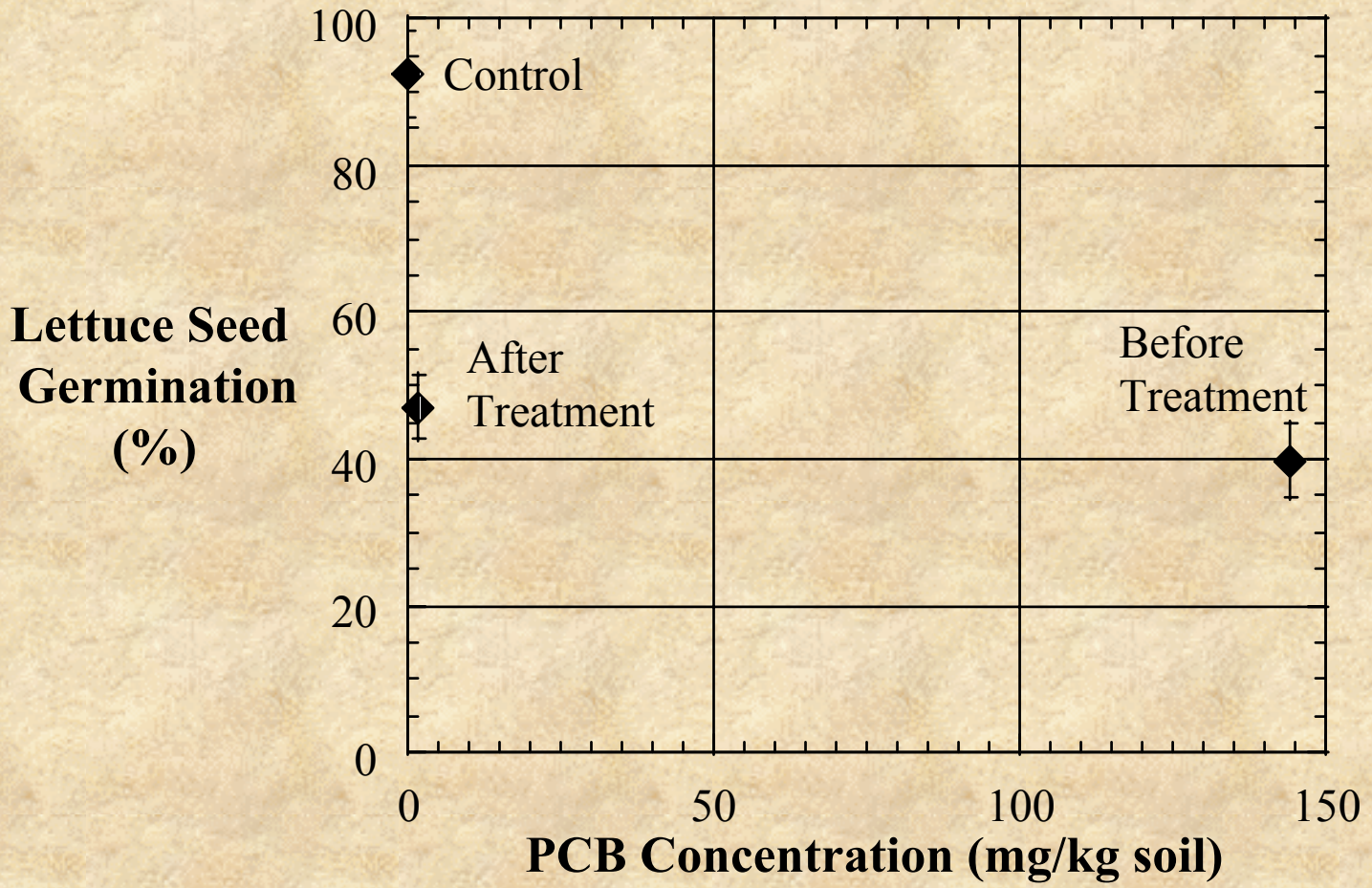
Case Study 1 - Bioassays

Bioassay	Organism	Exposure Matrix	Exposure Period	Endpoint
Earthworm Survival	E. fetida L. terrestris	soil	14 days	Survival
Seed Germination	Oats and Lettuce	soil	5 days	Survival
Earthworm Reproduction	E. fetida	soil	3 weeks	Survival, body mass, number of cocoons, cocoon hatchability
Root Elongation	Oats and Lettuce	soil	5 days	Growth
Allium Mitotic Aberrations	Allium	water extract	24 hour	Mitotic index, chromosomal abnormalities

Case Study 1 - Solvent Extraction



Case Study 1 - Results



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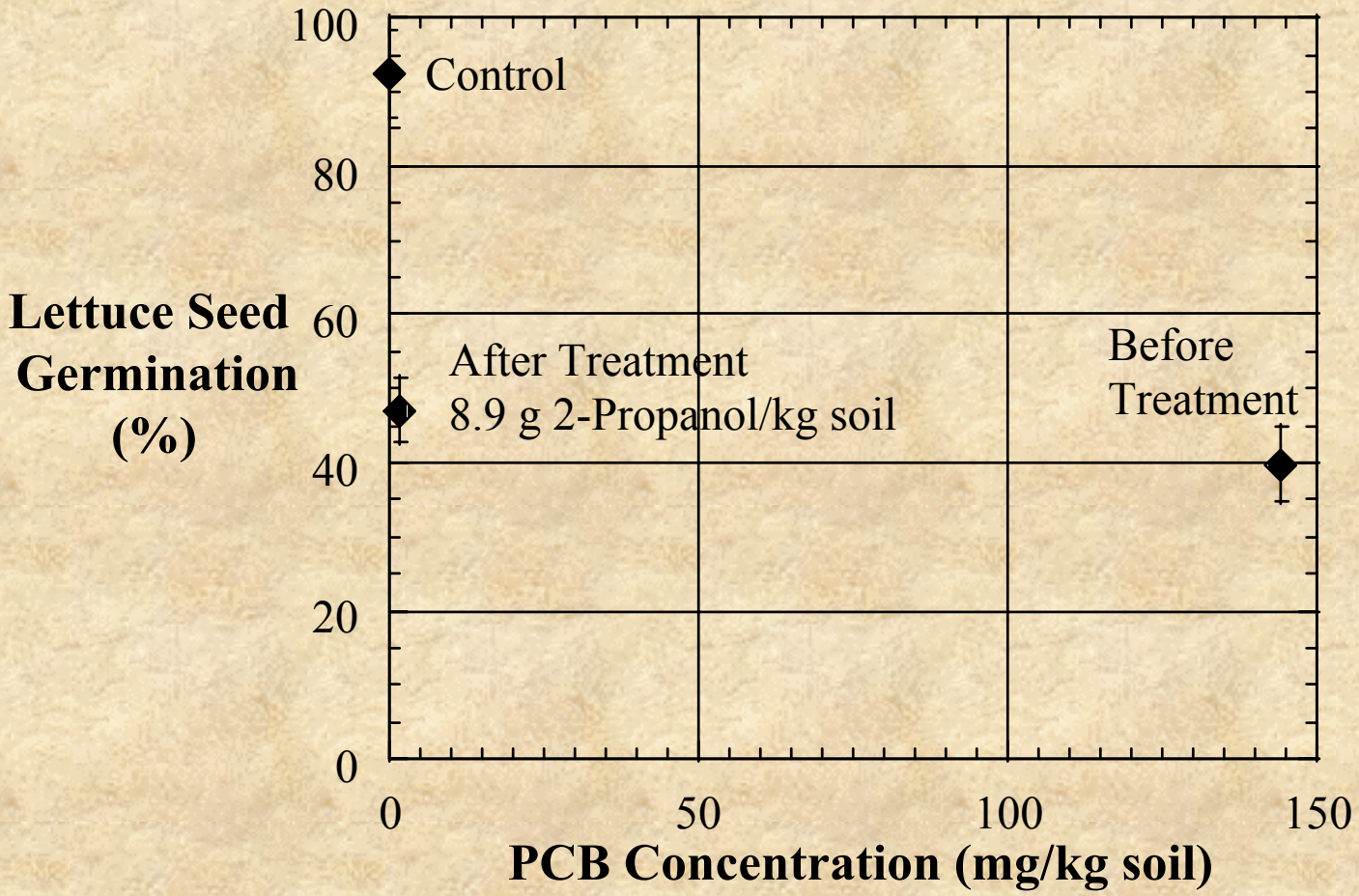
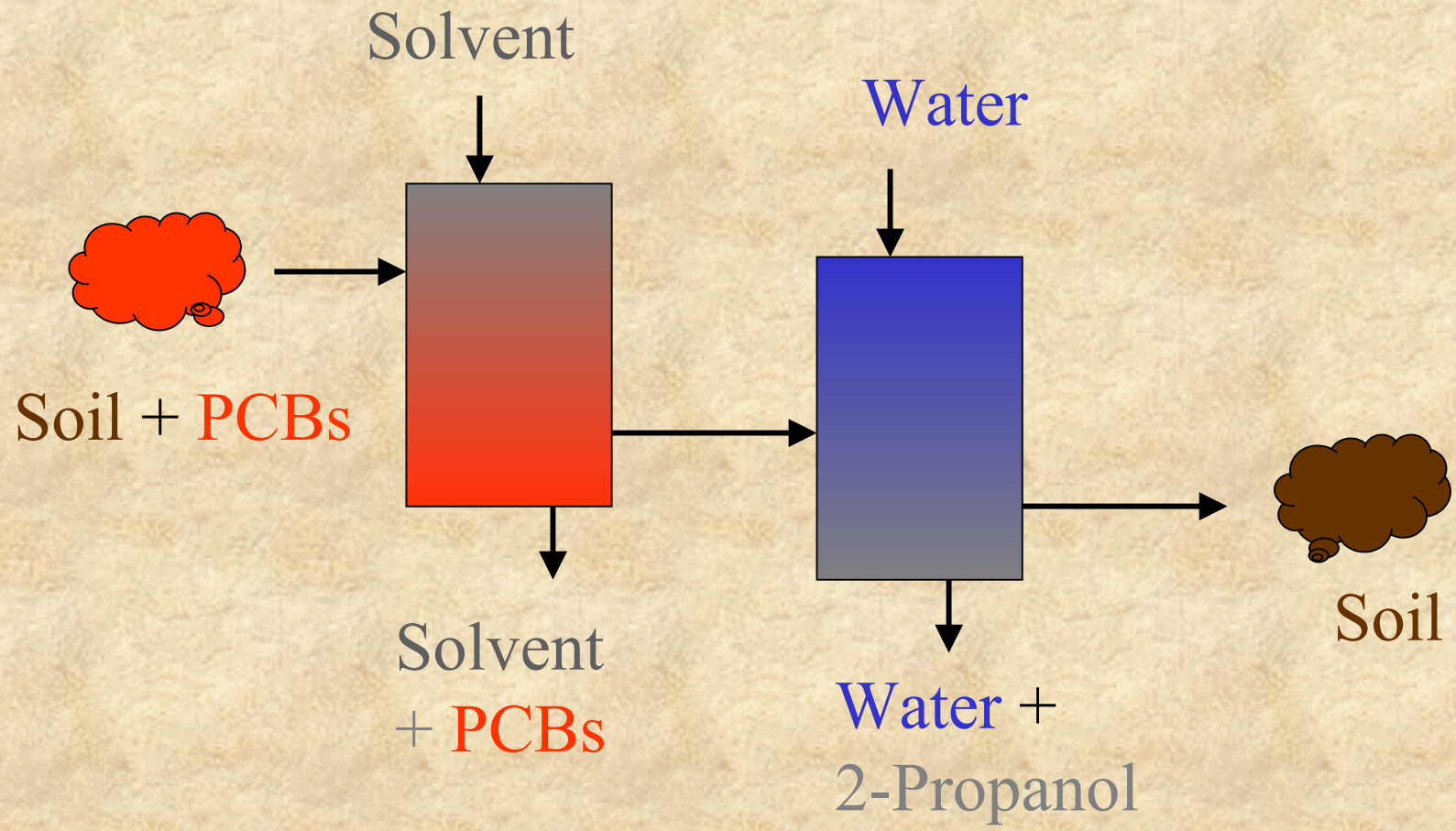
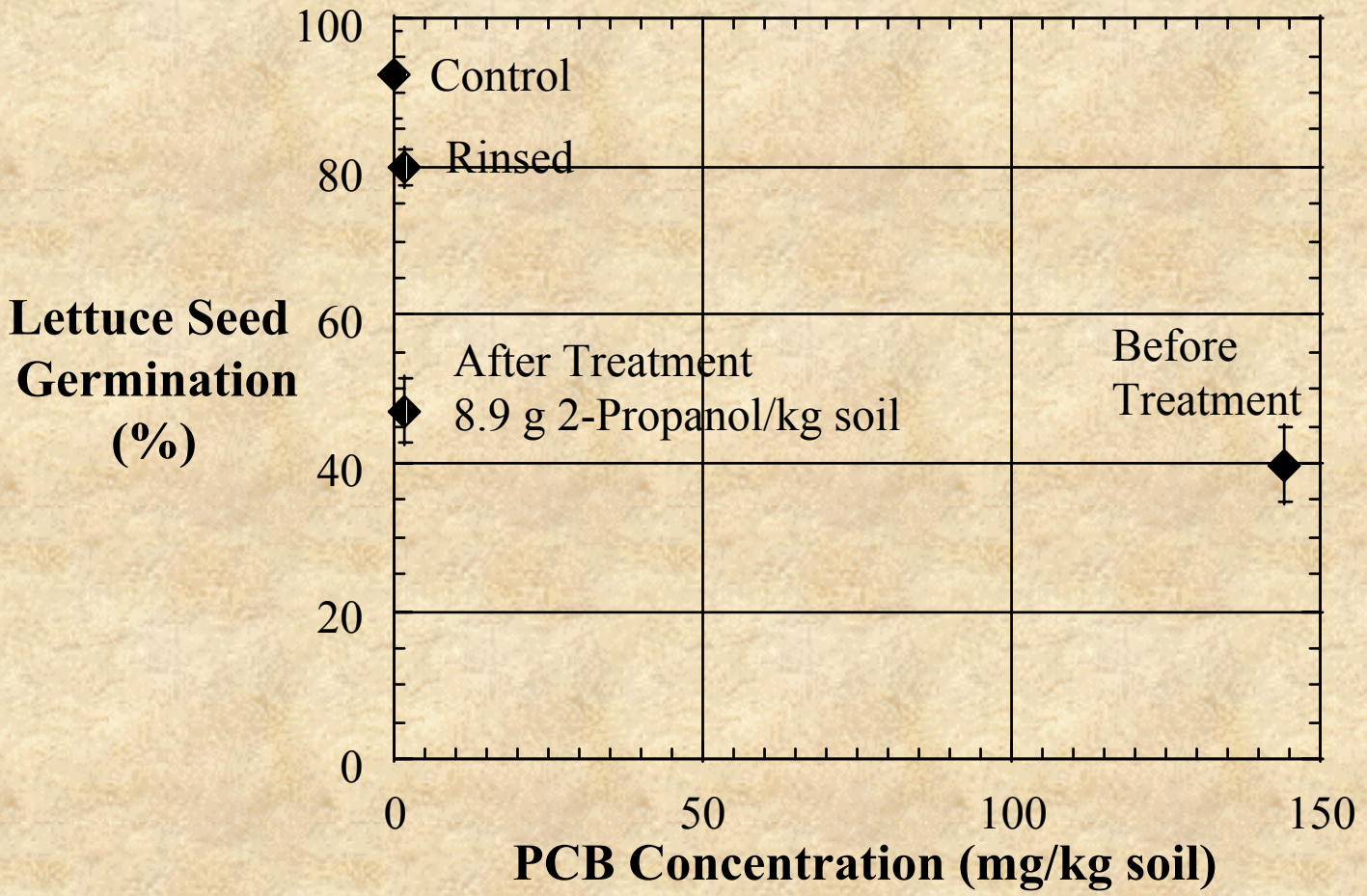


Case Study 1 - Better RM



Case Study 1 - Results 2



Case Study 1 - Summary

- Solvent extraction removed PCBs from soil
- Process residues were as toxic as PCBs
- Better RM - Add rinse step
  - Reduce PCB concentration
  - Reduce toxicity

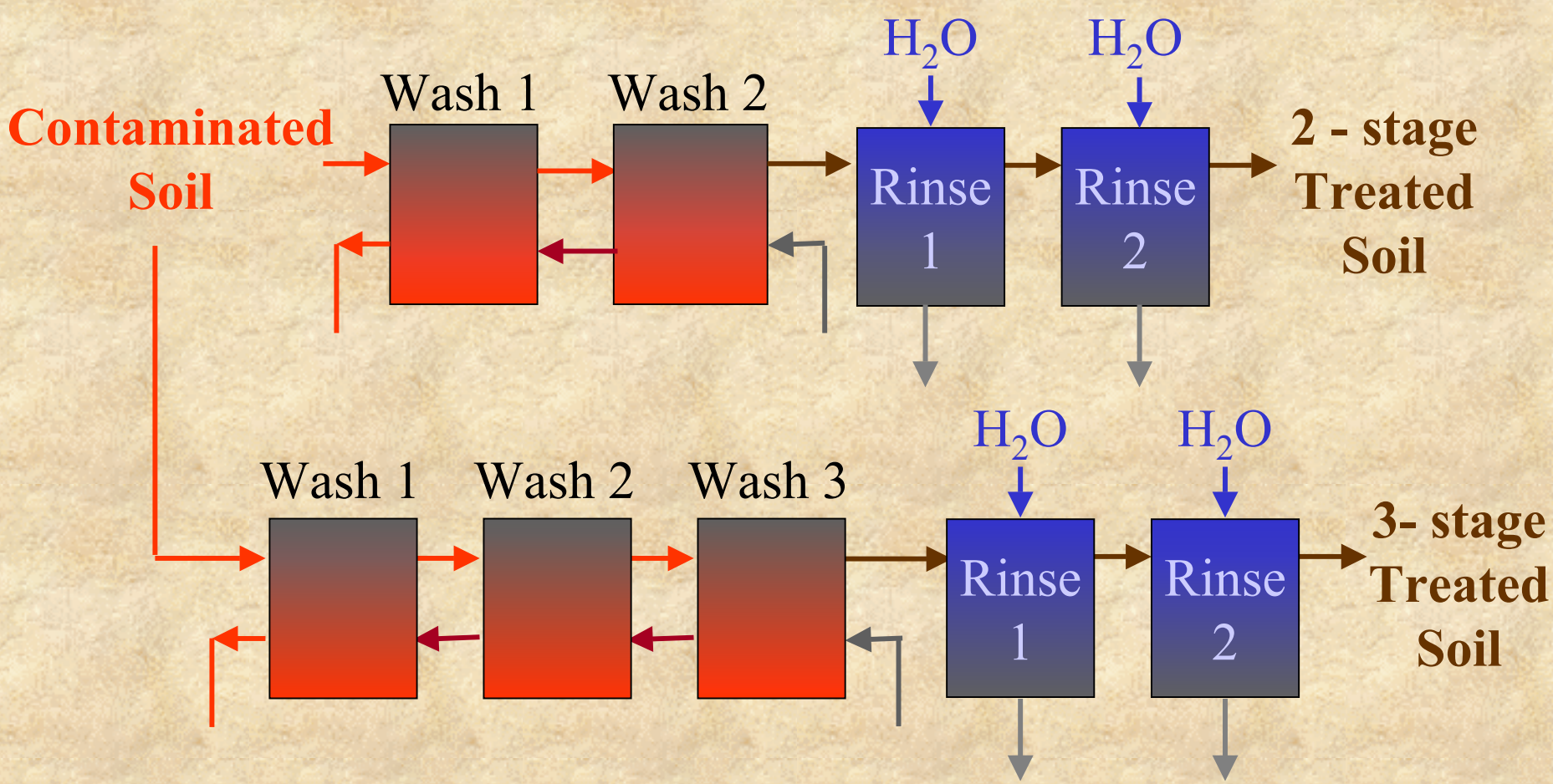
Case Study 2

- Remediation of soil contaminated with wood treating wastes by Soil Washing
  - Fluid - Ethanol-water mixture
  - Question - 2 or 3 Soil Washing stages?
- Principals
  - Soil Washing - Richard Brenner, Makram Suidan, George Sorial, Amid Khodadoust, Karen Koran, and Gregory Wilson
  - Ecotoxicity Evaluation - Carolyn Acheson, Jennifer Mansfield , Yonggui Shan, and Margaret Kupferle

Case Study 2

- Chemical Analysis
  - PCP
  - Hydrocarbons - alkanes and PAHs
- Bioassays
  - Earthworm Survival
  - Seed Germination and Root Elongation in Lettuce and Oats

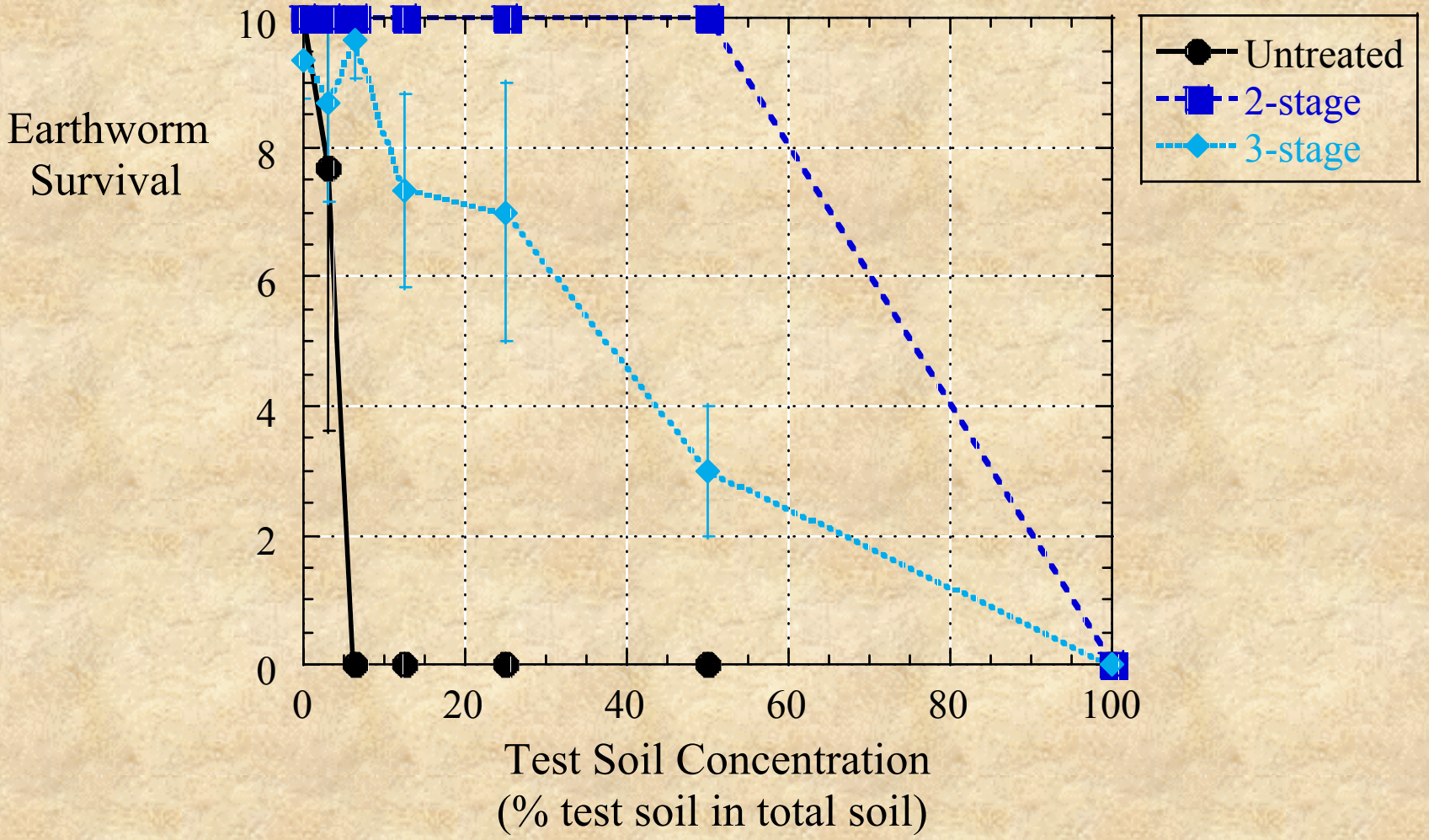
Case Study 2 - Soil Washing



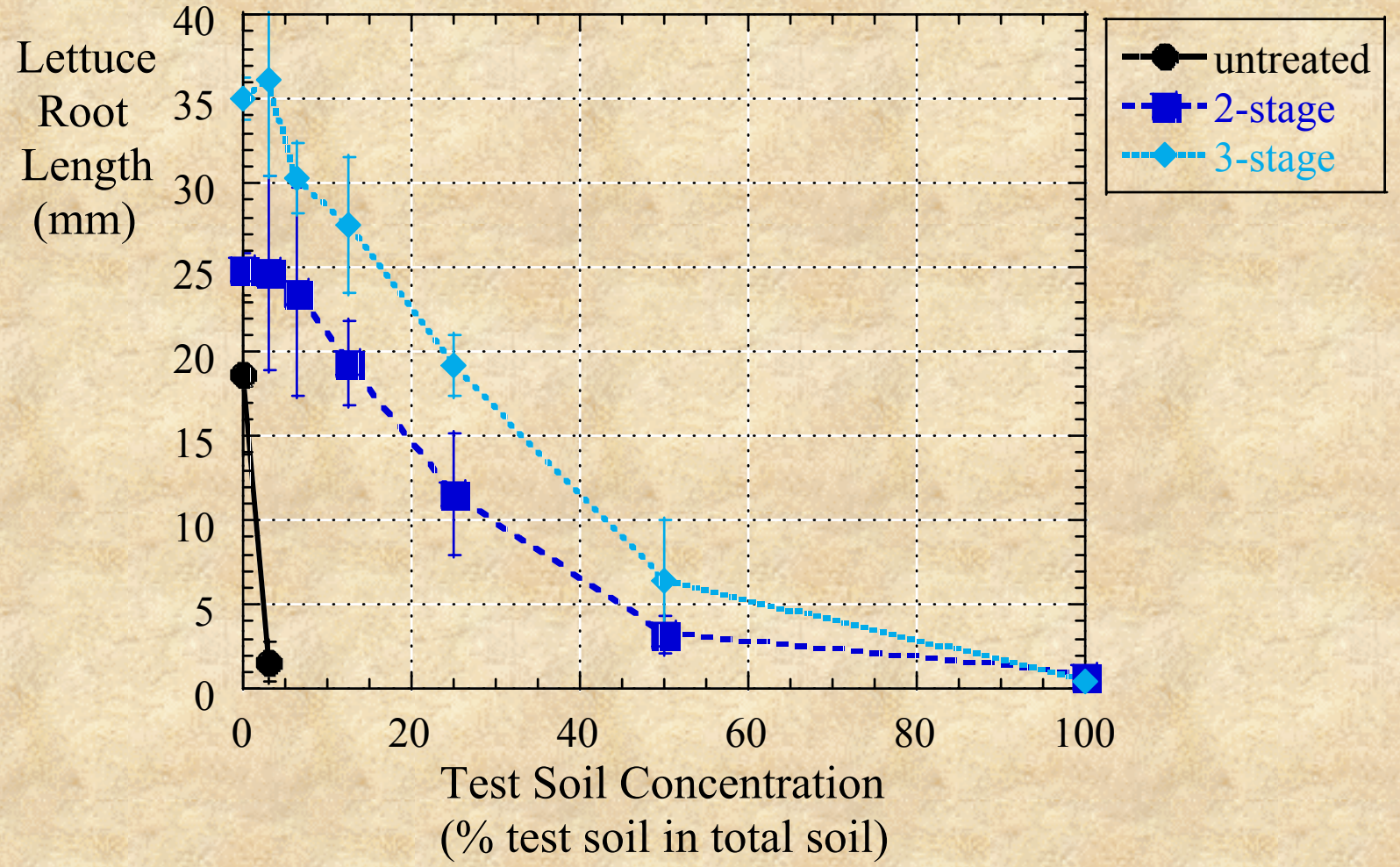
Case Study 2 - Results

Chemical	**Concentration (mg/kg dry soil)**		
	Untreated	2-stage	3-stage
PCP	950 ± 51	31± 1	9 ± 1
Alkanes	1761 ± 46	130 ± 17	59 ± 12
PAHs	494 ± 20	12 ± 2	<3

Case Study 2 - Results



Case Study 2 - Results



Case Study 2 - Results

Bioassay	Untreated	2-stage	3-stage
LC 50 (% test soil in total soil)			
Earthworm Survival	4.0	70.7	35.0 (26.3, 43.4)
Lettuce Seed Germination	5.1*	>100	89.4 (74.4, >100)
EC50 (% test soil in total soil)			
Lettuce Root Elongation	<3.1 *	17.9 (7.6, 23.0)	16.2 (12.6, 20.0)
Oat Root Elongation	12.1 (0, 23)	49.8 * (44.7, 55.7)	44.6 * (35.1, 61.4)

* Response in reference toxicant or negative controls were not in expected range

Case Study 2 - Summary

- Soil Washing was effective
  - Chemistry
    - PCP, Alkanes, and PAHs removed
    - 3- stage process most effective
  - Bioassays
    - Earthworms and plants show reduced toxicity in treated soils.
    - 2-stage process most ecologically hospitable
- Likely that soil washing alters other aspects of soil

Risk Management of EDCs

- Uncertainties of EDCs
  - Unknown endocrine activity of degradation products
  - Unknown effectiveness of treatments in reducing endocrine activity
- Concurrent chemical and biological measures of effectiveness recommended

EDC Bioassays - Considerations

- EDCs of concern in NRMRL projects
- Concentrations
- Environmental Matrices
- Data Quality - Reproducibility and Reliability
- Practicality - Cost and Ease of Use
- Recommendations of Others
- Adaptability to RM projects

EDCs of Concern in NRMRL projects

- Alkylphenols
- Chlorinated Dioxins and Furans
- Estrogens, biogenic and pharmaceutical
- PCBs

All are estrogenic;
some have thyroid and developmental effects

EDC Bioassays - Considerations

- Environmental Matrices in NRMRL projects
  - Air
  - Water
  - Solids - soils, sediments, and biosolids
- Concentrations
  - Water - as low as ng 17 β-estradiol/L
  - solids - levels vary

EDC Bioassays - Considerations

- Data quality
- Practicality
- Adaptability
- Sensitivity

Evaluated by

- Peer reviewed literature
- EDSTAC report
- ORD colleague recommendations

Types of EDC Bioassays Considered

- Sediment/Aquatic Invertebrate tests
- Terrestrial Invertebrate tests
- *In vitro* tests

Fish Vitellogenin mRNA assay through cooperation with MERB/NERL

Sediment/Aquatic Invertebrate tests

- Advantages
  - Commonly studied aquatic organisms
  - Many endpoint options
- Disadvantages
  - Mechanism of action - interference with molting controlled by steroid hormones, ecdysteroids
  - Require substantial lab equipment
  - Test duration - about 1 month

From www.AquacultureStore.com

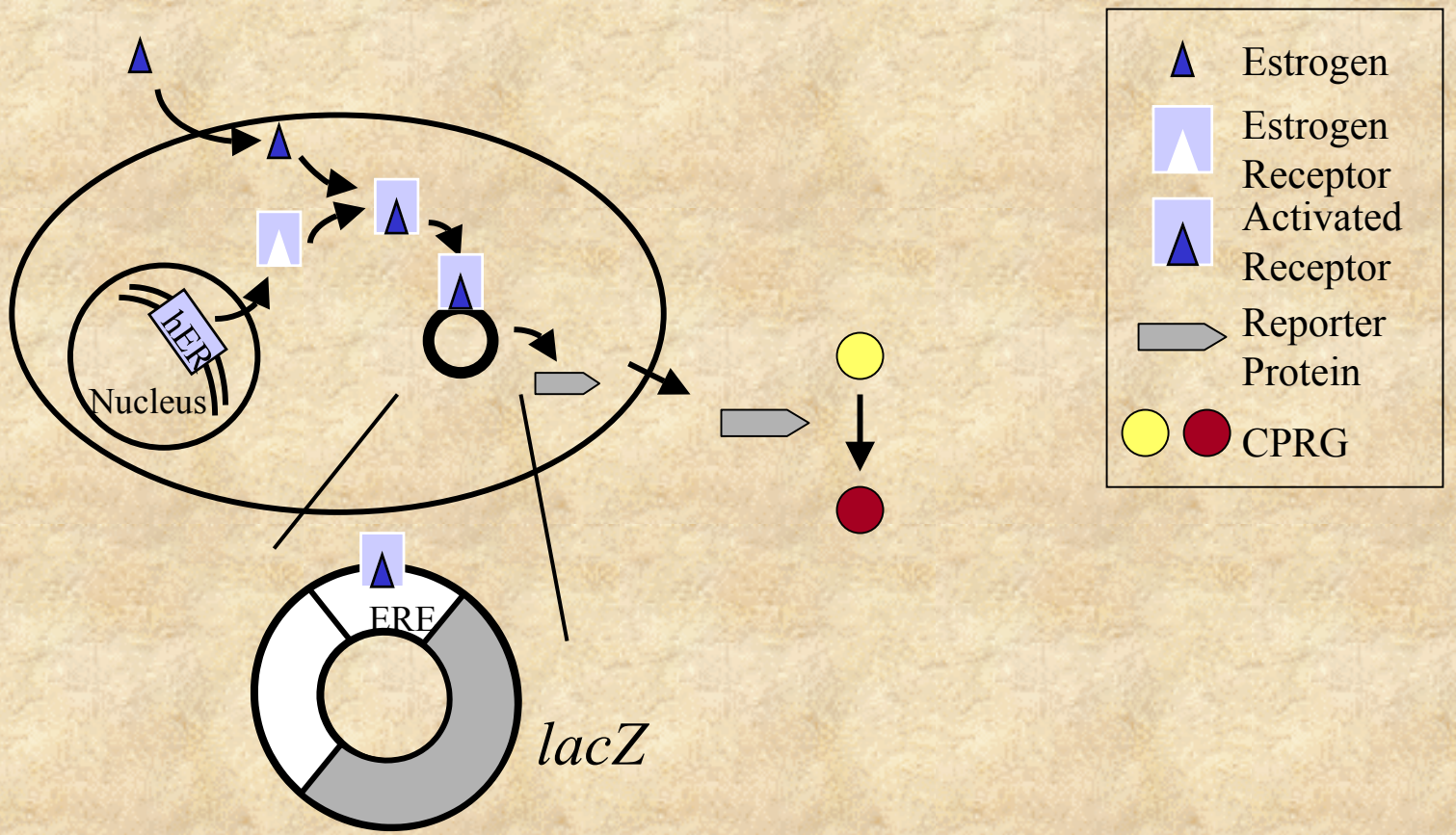
Terrestrial Invertebrate tests

- Imposex Occurrence - measure of androgenicity
- Earthworm Reproduction
  - methods exist
  - endpoints such as number of cocoons and number of hatchlings per cocoon
  - endpoints are not directly related to endocrine function
  - unknown sensitivity to EDCs of concern

In Vitro Assays

- Mammalian cells: E-Screen and MVLN
  - organism - immortal mammalian cell (MCF-7)
  - endpoint - proliferation or luciferase production
  - MVLN - recommended by EDSTAC
- Yeast Estrogen Screening Assay (YES)
  - evaluated by EDSTAC
  - commonly used in peer reviewed literature
  - not recommended for chlorinated pesticides
- Both - reported sensitivity at low concentrations

EDC Bioassay Selected for Adaption - YES Assay



From Routedge and Sumpter, 1996. Environ. Tox and Chem. 15: 241- 248

NRMRL Sponsored EDC RM Projects Using Bioassays

Project	**EDC**	**Prinicpal Investigator**	**Bioassay**
Evaluation of Drinking Water Treatment Techniques for EDC Removal	Steroid Hormones Alkyl Phenols	Kathleen Schenck	MVLN Assay
Potential of CAFOs to Contribute Estrogens to the Environment	Estrogens	Steven Hutchins	FETAX XTRA
Investigations of Sorption and Transport of Hormones and Animal Pharmaceuticals	Estrogens	Suresh Rao Carl Enfield	YES Assay
Evaluating the Fate of EDCs During Wastewater Treatment	Steroid Hormones Alkyl Phenols	Paul McCauley	YES Assay Vitellogenin mRNA Assay
EDCs from Combustion and Vehicular Emissions	PCBs Dioxins/Furans	Brian Gullett	Vitellogenin mRNA Assay
Natural Recovery of PCBs in Sediments	PCBs	Richard Brenner	Vitellogenin mRNA Assay

Using Bioassays in a Hypothetical EDC RM Project

- EDC of Concern - Phthalates
  - commonly used as a plasticizer in many household products (including food containers)
  - suspected to cause alterations in human sexual development
- RM Project - Find a replacement plasticizer for phthalates

Hypothetical EDC RM Project - Phthalate Replacement

- Use computer models to find substances with appropriate chemical and physical properties
- Lab Testing of leading candidates
  - chemical and physical testing to determine if acceptable substitute
  - bioassays to evaluate biological activity
- Look at production processes
  - Are production by-products likely to cause problems?
  - Test bulk chemical - chemical, physical, biological properties

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